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From the INTERNATIONAL BUREAU

To:

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Date of mailing (day/month/year)

23 September 1999 (23.09.99)

Applicant's or agent's file reference

39567 / JMD

IMPORTANT NOTIFICATION

International application No.

PCT/GB99/00820

International filing date (day/month/year)

17 March 1999 (17.03.99)

1. The following indications appeared on record concerning:

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3. Further observations, if necessary:

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4. A copy of this notification has been sent to:

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Date of mailing (day/month/year)

18 November 1999 (18.11.99)

International application No.

PCT/GB99/00820

Applicant's or agent's file reference

39567 / JMD

International filing date (day/month/year)

17 March 1999 (17.03.99)

Priority date (day/month/year)

18 March 1998 (18.03.98)

Applicant

ROSER, Bruce, Joseph et al

1. The designated Office is hereby notified of its election made:

☒

in the demand filed with the International Preliminary Examining Authority on:

15 October 1999 (15.10.99)

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in a notice effecting later election filed with the International Bureau on:

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 47/26, 47/22, A23L 1/275, A61K 7/00, 9/00	A1	(11) International Publication Number: WO 99/47174 (43) International Publication Date: 23 September 1999 (23.09.99)
(21) International Application Number: PCT/GB99/00820 (22) International Filing Date: 17 March 1999 (17.03.99) (30) Priority Data: 9805699.7 18 March 1998 (18.03.98) GB 9820689.9 23 September 1998 (23.09.98) GB (71) Applicant (for all designated States except US): CAMBRIDGE BIOSTABILITY LIMITED [GB/GB]; Sumper House, 8 Station Road, Histon, Cambridge CB4 4LQ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): ROSER, Bruce, Joseph [GB/GB]; 4 Archway Court, Barton Road, Cambridge CB3 9LW (GB). DE CASTRO, Arcadio, Garcia [ES/GB]; 86 Wulfstan Way, Cambridge CB1 4OH (GB). (74) Agent: DAVIES, Jonathan, Mark; Reddie & Grose, 16 Theobalds Road, London WC1X 8PL (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: AMORPHOUS GLASSES FOR STABILISING SENSITIVE PRODUCTS		
(57) Abstract		
<p>Disclosed is a method of drying, without damage, a compound which is subject to deactivation on drying, or a mixture of such compounds, comprising subjecting an aqueous system containing the compound or mixture to drying in the presence of one or more monosaccharide sugar alcohols and at least one additive which is a glass-former or a glass-formation-facilitator, whereby the compound solidifies from solution as an amorphous glass rather than by forming crystals.</p>		

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AMORPHOUS GLASSES FOR STABILISING SENSITIVE PRODUCTS

Drying of biological molecules such as foodstuffs, vaccines and drugs is an ancient way of preserving them. However, the price that often has to be paid for increased stability is product damage and a decrease in quality. More recently it has been learned that certain additives can prevent this damage. Levine and Slade were amongst the first to realise that the best additives were substances that tended to solidify from solution as an amorphous glass rather than by forming crystals (Levine H and Slade L., Principles of cryostabilisation technology from structure/property relationships of carbohydrate/water systems: A review. *Cryoletters* **9** 21-63 (1988)). Foremost amongst the stabilisers they recommended were certain sugars, notably sucrose. Although not formally proved, the stability of these dried formulations is assumed to be a function of the transformation of the sugar solution into a glass as it dries. As a consequence of the progressive increase in viscosity of the sugar syrup, it is thought that the active molecules are concentrated by a smooth progression from their mobile native state in liquid solution to an immobilised solid solution or glass. In the glass, molecular motion and hence diffusion-based molecular interactions are negligible. The chemistry responsible for degradation is arrested and the product remains stable as long as it remains dry and glassy (Franks F., Freeze drying: From empiricism to predictability. *Cryoletters* **11** 93-110 (1990)).

The simple view that glass formation is the explanation for stability is incomplete. This explanation only holds for glasses that are inherently chemically unreactive and stable. It is incontrovertible that certain substances that form physically excellent glasses are nevertheless very poor stabilisers. Many of these are either chemically reactive, such as reducing sugars or are unstable, like sucrose or sorbitol and break down to reactive intermediates which degrade the product during storage in the dry state (Newman Y.M., Ring S.G. and Colaco C. The role of trehalose and other carbohydrates in biopreservation. *Biotechnol. & Genet. Eng. Rev.* **11** 263-294 (1993)). A common reactivity of sugar glasses is the reducing action of the carbonyl group and the degradation products formed are often the familiar carbonyl-amine compounds of the Maillard reaction (Ellis G.P. The Maillard reaction. *Adv. Carbohyd. Chem.* **14** 63-134 (1959)). Because the Maillard reaction is temperature dependent, it is only slowly progressive at low temperatures. Many glass-forming materials give excellent preservation of activity during the drying process

itself but the product subsequently undergoes progressive deterioration unless stored under refrigeration.

The error of ignoring the subtle sugar chemistry that can proceed in dried preparations is widespread in the literature and has led to the advocacy of simple tests of the glass transition temperature of pure sugar solutions as a means of selecting good stabilisers. This approach has actually led to the recommendation of quite useless substances in the past (Franks F. Freeze drying: From empiricism to predictability *Cryoletters* **11** 93-110 (1990)). In fact, the efficacy of a formulation is the result of multiple physical and chemical interactions between all the components in the formulation, including the active, on drying. All these interactions may not be predicted by current theories.

The superiority of the disaccharide trehalose as a stabiliser was first indicated by its prevalence in certain rare living creatures, which regularly dried out and could come back to life on rehydration. (Crowe J.H., Crowe L.M. and Chapman D. Preservation of membranes in anhydrobiotic organisms: The role of trehalose. *Science* **223** 701 (1984)). In laboratory studies, trehalose incorporated into the buffer solutions from which active biomolecules were dried, resulted in a product with quite remarkable resistance to denaturation by heat (Colaco C.A.L.S., Sen S., Thangavelu M., Pinder S. and Roser B. Extraordinary stability of enzymes dried in trehalose: Simplified molecular biology. *Biotechnol.* **10**. 1007-1011 (1992)). Because it is subsequently degraded in the body by the specific enzyme, trehalase, to two molecules of glucose, trehalose possesses many of the properties of the ideal industrial stabiliser for foods and medical products. A large scientific and patent literature has now developed on trehalose stabilisation of foods, vaccines, diagnostics and drugs. The disadvantages of trehalose are that it is as yet not approved by the regulatory authorities, it is expensive and it contains contaminating reducing sugars, especially glucose, in all but the most rigorously purified material.

One of the alternative non-reducing and chemically stable sugar derivatives that might be expected to stabilise effectively is mannitol. Because of its remarkable resistance to water sorption at high atmospheric humidity (Wade A. and Weller P.J. Handbook of Pharmaceutical Excipients second edition p296 (1994)), it is widely used in tablet and powder formulations as a bulking and anti-caking agent. In combination with other excipients such as glycine, it is also widely used in freeze dried parenteral preparations but

is added as a carrier to produce a stiff, homogeneous cake that improves the appearance of the lyophilised plug in a vial (Wade & Weller The Excipients Handbook 1994).

In published surveys of the stabilising ability of a wide range of sugars and sugar derivatives (Colaco C.A.L.S., Smith C.J.S., Sen S., Roser D.H., Newman Y., Ring S. and Roser B.J. Chemistry of protein stabilisation by trehalose in *Formulation and delivery of proteins and peptides* Cleland and Langer eds American Chemical Society Washington 222-240 (1994)), it was shown that mannitol and sorbitol were very poor stabilisers. Indeed in several patent applications it has been claimed that mannitol and certain other monosaccharide alcohols cannot stabilise at all. PCT No WO 91/18091 "Stabilisation of biological macro-molecular substances and other organic compounds", Roser B.J. and Colaco C claimed that only non-reducing glycosides of a polyhydroxy sugar alcohol or other straight chain polyalcohol or raffinose, stachyose or melezitose were effective in achieving stability, especially on storage. This patent states "Thus the monosaccharide sugar alcohols galactitol, mannitol and erythritol are not satisfactory protective agents". US patent Number 5,621,094 "Method of Preserving Agarose Gel Structure During Dehydration by Adding a Non-reducing Glycoside of a Straight Chain Sugar Alcohol" by Roser B. and Colaco C states that "glucose mannitol and sorbitol failed after one week" while "lactitol and trehalose were perfect after >12 weeks". It further defined effective formulations as "wherein the non-reducing glycoside of a straight chain sugar alcohol does not form crystals during dehydration". PCT No WO 96/05809 "Improved method for stabilisation of biological substances during drying and subsequent storage and compositions thereof", by Colaco C., Roser B.J. and Sen S. claims methods wherein even reducing sugars are used to stabilise products. This is achieved by preventing the sugars from attacking the product by using Maillard reaction inhibitors. This application states that mannitol has no stabilising effect whatsoever.

We have now found this to be incorrect. In contradiction to the statements in these documents we have found that certain monosaccharide sugar alcohols such as mannitol and inositol can be excellent stabilisers when correctly formulated and in fact have significant advantages over trehalose for some applications. In view of mannitol's acceptance by regulatory authorities and widespread use in the healthcare industry in both parenteral and oral formulations, it has considerable advantages as a new stabilising excipient. Its low cost and chemical inertness, together with its exceptional stability and

its high purity and safety, would make it the stabiliser of choice for pharmaceuticals. We have found that certain substances must be present in a formulation to convert mannitol into an excellent stabiliser. The effect of these substances is dose dependent and below a threshold concentration they do not work. The substances useful in accordance with this invention promote the drying of mannitol solutions as glasses rather than crystals. One of the most potent materials is the borate ion either as boric acid, or tetraborate salts of sodium or potassium. This probably forms a network complex with mannitol or even a covalent compound, sodium mannitoborate. Subsequent to the filing of this application containing the disclosure of the surprising efficacy of small amounts of borate in inducing glass formation in drying mannitol solutions, similar beneficial effects with borate and trehalose were reported by Miller et al. Notably, the molar ratio of borate to trehalose used by these authors was considerably higher than we found to be necessary. (Miller DP, Anderson RE and de Pablo JJ. Stabilisation of lactate dehydrogenase following freeze-thawing and vacuum-drying in the presence of trehalose and borate. *Pharmaceutical Research* 15 1215-1221 (1998)). Other effective materials such as calcium lactate, and proteins such as serum albumin or gelatin, or polyamine materials such as polyvinyl pyrrolidone, or polyvinyl alcohol, intrinsically form glasses themselves when dried from solution. Yet other effective chemicals such as acetate salts, will form glasses but only when quenched from the melt and only when the melt contains several metal cations rather than a single cation, such as sodium and calcium. An additional property of the materials identified to date is that the beneficial actions of these materials are additive so that they can be mixed together in successful formulations which contain sub-threshold doses of each additive alone. Other substances which are either themselves glass-formers (under certain conditions) or are glass-formation-facilitators such as the phosphate salts of sodium and potassium and sodium silicate are capable of being utilised to make stabilising glasses according to this invention.

The quality of the glasses made by this process is high. The glass transition temperature (T_g) of 1:1 w/w mannitol/calcium lactate glass is around 68°C (Figure 1). This compares with a T_g of around 90°C for a trehalose/sodium sulphate glass dried under the same conditions (Figure 2). Both types of glass have T_g's well above any possible ambient storage temperature and, because the glasses are chemically inert and non-reactive, the entrapped products are stable at room temperatures and require no refrigeration of any kind.

It is a particular advantage of this invention that sugars which have previously been known as stabilisers in methods of freeze drying may be used successfully in drying compounds subject to deactivation on drying whilst utilising drying temperatures above freezing point (e.g. room temperature or above). Thus US Patent 5,589,167 "Excipient stabilisation of polypeptides treated with organic solvents" by Cleland and Jones reports that mannitol and trehalose are both excellent stabilisers for the recombinant peptides human Growth Hormone and human Interferon gamma on freeze-drying and on exposure to organic solvents. These authors found that the ratio of active to excipient was critical in achieving the optimum stabilisation. Without wishing to be bound by theory, it is likely that the good results reported in this patent are not so much due to the substitution of freeze-drying for ambient temperature drying but the use of very high ratios of active to excipient in the formulations. Under these circumstances, where the mass ratio of peptide to mannitol or trehalose was 1 to 1 or higher, the peptide itself was acting as an enhancer of glass transformation of the drying sugar solutions in a manner analogous to the action of albumin or gelatin shown below. A serious disadvantage of this approach is that highly potent drugs and vaccines need tiny amounts of stabilising sugars to give a good stabilising glass. Single dose ampoules for example appear to be completely empty; the thin film of dried product being invisible in the container. This is confusing to the end user and can be wasteful, if the container is discarded in error, or even dangerous, if the time to complete dissolution of the product is not obvious after reconstitution. This greatly reduces the flexibility of formulation and presentation of the product.

What is required is a robust formulation that inherently forms a good glass even in the absence of product but which can accommodate a wide range of product concentrations without loss of glass forming capacity and stabilising efficacy. A number of sugar alcohols previously rejected as stabilising agents in the prior art listed above such as mannitol, xylitol, inositol, arabinitol and galactitol stabilise very effectively when correctly formulated so as to promote the formation of a glassy matrix, rather than crystals, on drying. A simple method for inhibiting crystallisation is to mix two or more sugars or sugar derivatives together in the same formulation. When correctly chosen, these mutually inhibit crystallisation and the mixture dries as an amorphous glass. In some cases these glasses are more robust on storage and give greater stability to an included product than trehalose itself.

Brief Description of the Figures

Figure 1 shows differential scanning calorimetry of a 50 / 50 w/w mannitol / calcium lactate glass showing a clear glass transition at a temperature of 68 °C;

Figure 2 shows differential scanning calorimetry of a 50 / 50 w/w trehalose / calcium lactate glass showing a clear glass transition at a temperature of 90 °C;

Figure 3 shows the percentage recovery of alkaline phosphatase activity after vacuum-drying in either trehalose or formula 7 containing mannitol, inositol, galactitol and degraded gelatin (Byco C) followed by storage at 37°C or 50°C for up to 6 weeks. There is no loss with formula 7 but serious losses with trehalose;

Figure 4 shows the percentage recovery of alkaline phosphatase activity after freeze-drying in either trehalose or formula 7 containing mannitol, inositol, galactitol and degraded gelatin (Byco C) followed by storage at 37°C or 50°C for up to 7 weeks. There is little loss with either stabiliser;

Figure 5 shows the percentage recovery of Erythropoietin (EPO) after vacuum-drying in either trehalose or formula 8 containing mannitol, inositol, galactitol and calcium lactate followed by storage at 37°C or 50°C for up to 6 weeks. While there is serious losses with trehalose, no loss occurs with formula 8; and

Figure 6 shows the percentage recovery of EPO after freeze-drying in either trehalose or formula 7 containing mannitol, Inositol, galactitol and degraded gelatin (Byco C) or formula 8 in which calcium lactate was substituted for the gelatin. After storage at 37°C or 50°C for 7 weeks, there is no loss with any of the stabilising formulations.

Figure 7 shows the percentage recovery of alkaline phosphatase activity after spray drying formula 9 to which had been added insoluble calcium phosphate powder to increase the density of the glass microspheres. After storage at either 37°C or 55°C for up to 90 days there was no significant loss of activity.

Figure 8 shows the percentage recovery alkaline phosphatase activity after spray drying formula 11 to which had been added insoluble barium sulphate powder to increase the density of the glass microspheres. After storage at either 37°C or 55°C for up to 90 days there was again no significant loss of activity.

Examples

Example 1

A solution of mannitol in water 20% w/v was pipetted in 100 µl volumes on to the surface of clean glass microscope slides which were laid flat on a hotplate at 70°C for drying. Within about 5 min. the solution had dried into a mass of crystals. A 20% solution of trehalose or palatinit, dried under the same conditions formed a hard and transparent perfect glass film. Only the latter sugars stabilise actives successfully as described in the patents and publications referenced above. This is considered to be a function of their ability to form amorphous glass on drying.

The addition of a network forming additive such as sodium or potassium tetraborate to the mannitol solution in amounts of less than 10% of the weight of mannitol, completely inhibited crystallisation on drying and resulted in the formation of glasses as perfect as those made with trehalose or palatinit. This demonstrated that mannitol can form glasses under appropriate conditions and a search was then made for less toxic additives to achieve the same effect.

Example 2

Equal weights of trehalose or palatinit were mixed with the mannitol in solution and dried as above. In both cases this yielded perfect glasses showing that these two glass-forming sugars could inhibit the crystallisation of mannitol. Even a sugar which was not itself a glass former, such as galactitol, inhibited the crystallisation of a mannitol / inositol mixture which itself crystallised readily. Similar results were found when equal weights of other glass forming substances such as calcium lactate, albumin, polyvinyl pyrrolidone or degraded gelatin (Byco C) were added to mannitol in solution. To establish the longer term stability of these glasses they were held at 70°C overnight and then at room temperature and ambient humidity for several weeks and inspected frequently. All the above glasses were stable under both sets of conditions. When other monosaccharide alcohols such as galactitol, xylitol, arabinitol, adonitol, or inositol were substituted for

mannitol, similar results were obtained but the resulting glasses were very soft when alcohols of the pentose sugars were used.

Example 3

More complex mixtures of the monosaccharide alcohols could also be blended together with glass forming substances to yield excellent glasses, which showed good physical stability in the glass phase at 70°C and at ambient conditions for many weeks as described in Example 2. Some good formulations are:-

1. mannitol 33.3%, inositol 33.3% and PVP 33.3%
2. mannitol 31.6%, inositol 31.6% xylitol 5% and calcium lactate 31.6%
3. mannitol 33.3%, inositol 33.3% and calcium lactate 33.3%
4. mannitol 33.3%, inositol 33.3% and Byco C 33.3%
5. mannitol 23.3%, inositol 23.3% calcium lactate 30% and PVP 23.3%
6. mannitol 33.3%, arabinitol 33.3% and calcium lactate 33.3%
7. mannitol 30%, inositol 15% galactitol 15% and Byco C 40%
8. mannitol 30%, inositol 15% galactitol 15% and calcium lactate 40%
9. mannitol 33%, Byco C 33% and calcium lactate 33%
10. mannitol 50%, and Kollidon 30 (polyvinylpyrrolidone (PVP)) 50%
11. mannitol 33%, Kollidon 30 (polyvinylpyrrolidone (PVP)) 33% and calcium lactate 33%
12. mannitol 50%, and Dextran 50%
13. mannitol 33%, Dextran 33% and calcium lactate 33%

In short, simple trial and error experimentation will establish a successful formulation from mixtures of monosaccharide alcohols and a glass forming substance. By this method the final concentration of any single ingredient can be kept low. In this way a substantial total solids content can be achieved, even including sugar alcohols, which are individually not very soluble. The high solids content shortens drying times and increases the protection of the active during drying.

In addition to heat-assisted air-drying as above, formulations of this kind have been successfully vacuum dried, spray dried and freeze-dried.

Example 4

Stability of alkaline phosphatase enzyme.

Affinity purified alkaline phosphatase from bovine intestinal mucosa (Sigma Chemical Co cat No. p-8647) was vacuum-dried or freeze-dried in 200 μ l volumes in formulation Number 7 above or in trehalose, and stored at 37°C or 50°C for 5 weeks. Samples were tested for activity at intervals using the Sigma assay with p-nitrophenyl phosphate as substrate. Vacuum drying was done at a shelf temperature of 40°C and a vacuum of 30-100 millitorr for 4 hr. The temperature was then ramped gradually to 60°C over 1 hr and the vials were stoppered and removed from the vacuum chamber for high temperature storage trials. Freeze-drying was done in a Labconco dryer at an initial shelf temperature of -40°C for 3 hr at a vacuum of 30-100 millitorr. The shelf temperature was then ramped to 0°C at 5°C / min and held for 1 hr. The shelf temperature was then raised to 40°C at 5°C / min and secondary drying was continued for a further 3 hr when the vials were stoppered under vacuum and removed for storage trials.

While there was some variability in the assays of enzyme activity, obvious trends were observed. Samples dried by either method without stabilisers lost all activity within a day or two of storage (not shown). Samples dried in either mannitol alone or a modified formula 7 lacking the glass forming facilitator lost between 75 and 80% of activity within 3 days at 37°C.

There was also a progressive loss of enzyme activity seen with the samples vacuum-dried in trehalose, which was not seen in the samples dried in formula 7 (Fig 3). This was particularly marked where the samples had been stored at the higher temperature (50°C). No such loss in the trehalose-dried samples was seen in the freeze-drying experiments where trehalose appeared to be slightly superior to the monosaccharide alcohols (Fig 4). This result might possibly indicate that a higher residual moisture content may have been responsible for the serious losses with trehalose in the vacuum dried samples. Whatever the explanation, it is clear that formula 7 gives results which are equivalent to, or superior to, the results obtained with trehalose.

Example 5

Stability of recombinant human Erythropoietin (EPO)

EPO was vacuum dried or freeze-dried as above in the same solutions and also in a variant of formulation 7 in which calcium lactate was substituted for Byco C (Formula 8), and then subjected to the same stability tests before being assayed by a standard 2-site sandwich Enzyme Immunoassay.

The results again showed a serious, progressive deterioration in the vacuum dried samples dried in trehalose, more dramatic at 50°C storage temperature, which was not seen with the samples dried in formula 8 (fig 5). The deterioration in trehalose was not seen in the freeze-dried samples stored at 37°C (not shown) or 50°C (Fig 6). Irrespective of whether formula 7 or 8 was used, all samples showed essentially complete recovery of activity.

Example 6

The fluorescent protein R-Phycoerythrin was air-dried in trehalose, formula 3 or formula 4 on a hotplate as described in Example 1. The intensity of fluorescence was gauged visually when illuminated with a UV lamp. In the controls dried in trehalose fluorescence was retained. The material dried in formula 3 was masked by an intense silver autofluorescence from the Byco C while the material dried in formula 4 fluoresced with the characteristic orange colour with apparently undiminished intensity.

Claims

1. A method of drying, without damage, a compound which is subject to deactivation on drying, or a mixture of such compounds, comprising subjecting an aqueous system containing the compound or mixture to drying in the presence of one or more monosaccharide sugar alcohols and at least one additive which is a glass-former or a glass-formation-facilitator, whereby the compound solidifies from solution as an amorphous glass rather than by forming crystals.
2. A method according to claim 1 in which the aqueous system contains from 0.05 to 90% by weight of sugar alcohol.
3. A method according to claim 1 in which the ratio of sugar alcohol plus additive to compound is at least 0.25:1 preferably 0.5:1 by weight.
4. A method according to any of claim 1 to 3 in which the compound is a protein, polysaccharide or nucleic acid.
5. A method according to claim 4 in which the compound or mixture comprises an enzyme, serum, serum complement, an antibody or antigen (either free or coupled to a support), a nucleic acid, a fluorescent protein, or a vaccine component.
6. A method according to any of claim 1 to 5 in which the system is dried under conditions selected from one or more of the group consisting of ambient temperature or above, chill drying, freeze drying, spray drying, vacuum drying and drying at atmospheric pressure.
7. A dried product which is an amorphous glass containing monosaccharide sugar alcohol and at least one additive which is a glass-former or a glass-formation-facilitator and a compound which is subject to deactivation on drying, or a mixture of such compounds, in a weight ratio of sugar alcohol plus additive to compound of at least 0.25:1 preferably 0.5:1, the product having been dried.

8. A dried product according to claim 7 in which the compound is a protein, polysaccharide or nucleic acid.
9. A dried product according to claim 8 containing an enzyme, serum complement, an antibody or antigen (either free or coupled to a support), a nucleic acid, a fluorescent protein, or a vaccine component.
10. A method or product according to any preceding claim wherein the sugar alcohol is selected from the group consisting of mannitol, galactitol, xylitol, arabinitol and inositol.
11. A method or product according to any preceding claim wherein there is one or a mixture of additives selected from the group consisting of peptide, protein, borate ion, calcium lactate, phosphate, silicate and acetate salts.
12. A method or product according to claim 11 wherein at least one additive is selected from the group consisting of boric acid, tetraborate salt of sodium or potassium and sodium mannitoborate.
13. A method or product according to any preceding claim wherein the amorphous glass is formed from a mixture of 2 or more monosaccharide sugar alcohols.
14. A method or product according to any preceding claim wherein there is an additive which is a protein or denatured protein.
15. A method or product according to claim 1 or 7 wherein the amorphous glass is formed from a formulation having essentially a composition selected from:
 1. mannitol 33.3%, inositol 33.3% and PVP 33.3%
 2. mannitol 31.6%, inositol 31.6% xylitol 5% and calcium lactate 31.6%
 3. mannitol 33.3%, inositol 33.3% and calcium lactate 33.3%
 4. mannitol 33.3%, inositol 33.3% and Byco C 33.3%
 5. mannitol 23.3%, inositol 23.3% calcium lactate 30% and PVP 23.3%
 6. mannitol 33.3%, arabinitol 33.3% and calcium lactate 33.3%
 7. mannitol 30%, inositol 15% galactitol 15% and Byco C 40%

8. mannitol 30%, inositol 15% galactitol 15% and calcium lactate 40%
9. mannitol 33%, Byco C 33% and calcium lactate 33%
10. mannitol 50%, and Kollidon 30 (polyvinylpyrrolidone (PVP)) 50%
11. mannitol 33%, Kollidon 30 (polyvinylpyrrolidone (PVP)) 33% and calcium lactate 33%
12. mannitol 50%, and Dextran 50%
13. mannitol 33%, Dextran 33% and calcium lactate 33%

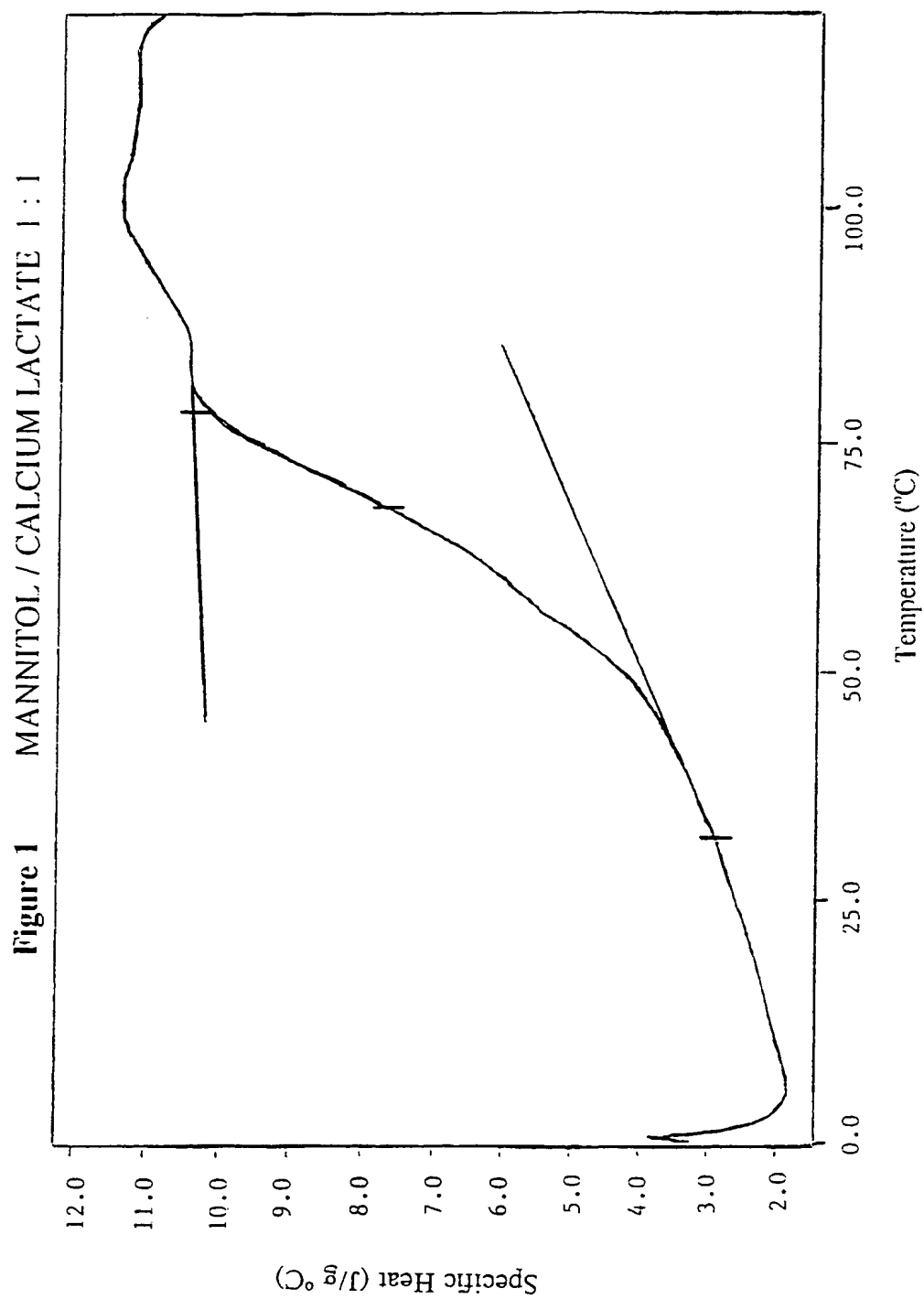


Figure 2 TREHALOSE / CALCIUM LACTATE 1 : 1

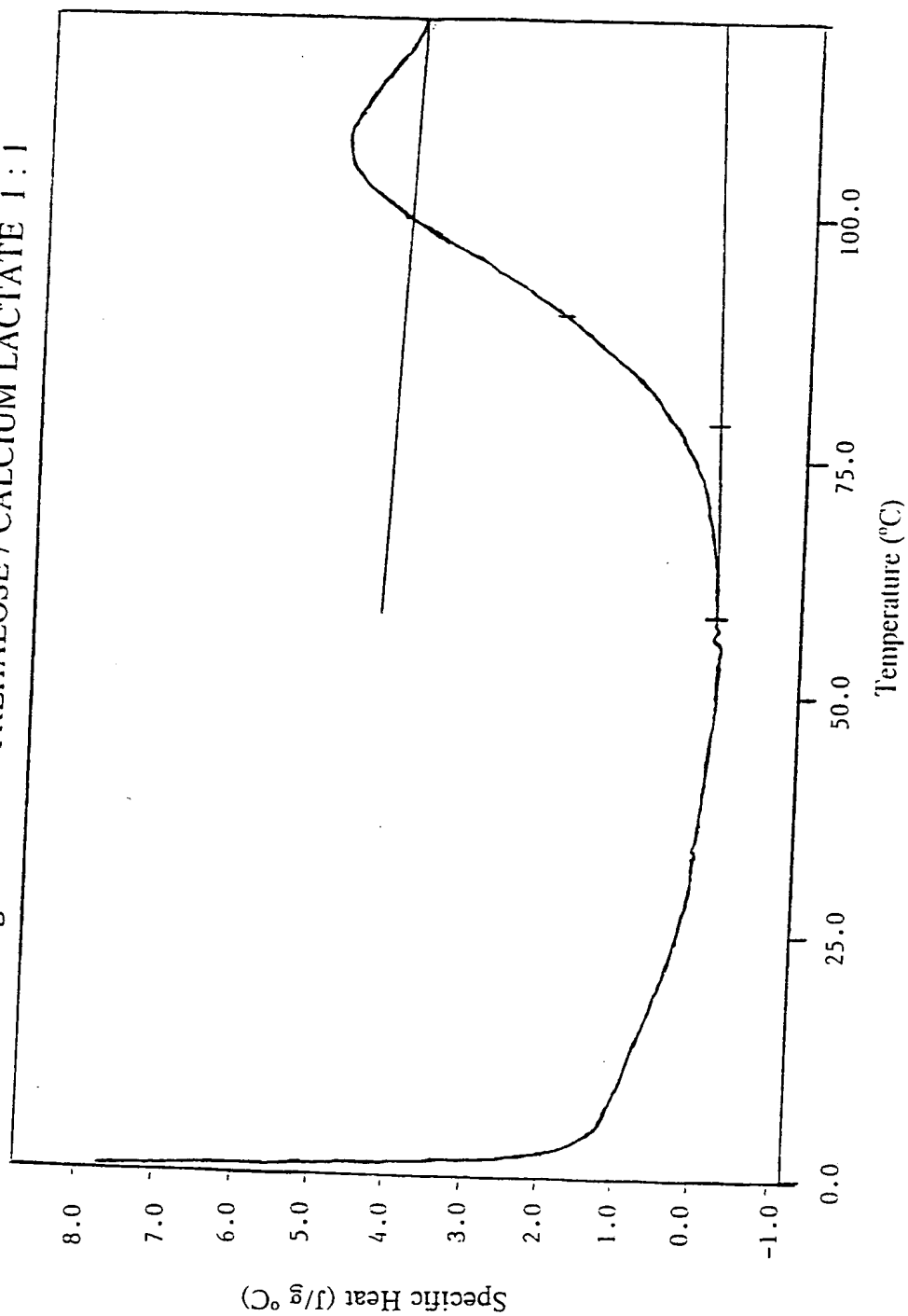
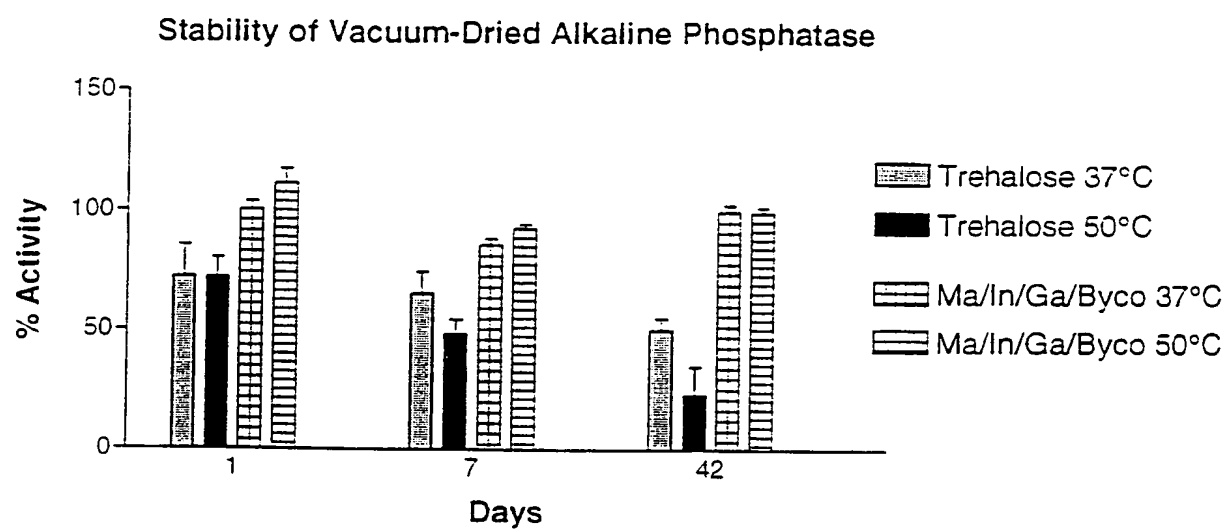
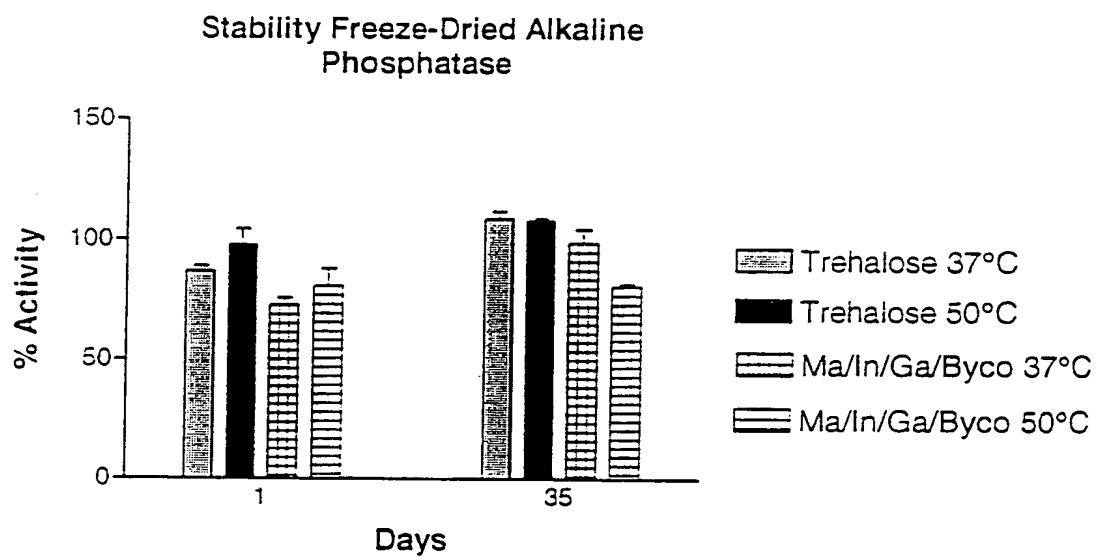


Figure 3



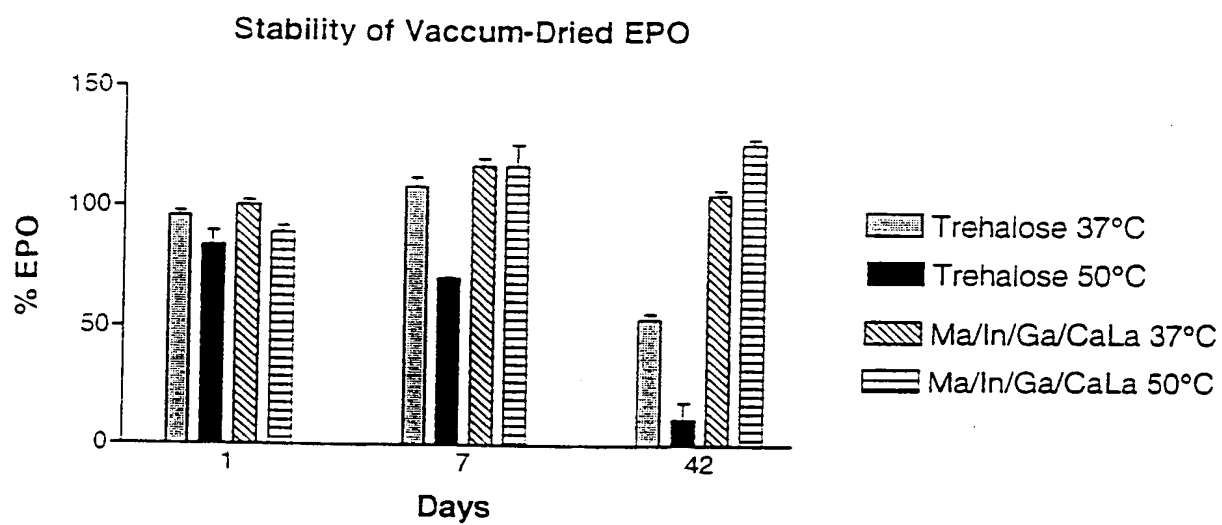
4/8

Figure 4



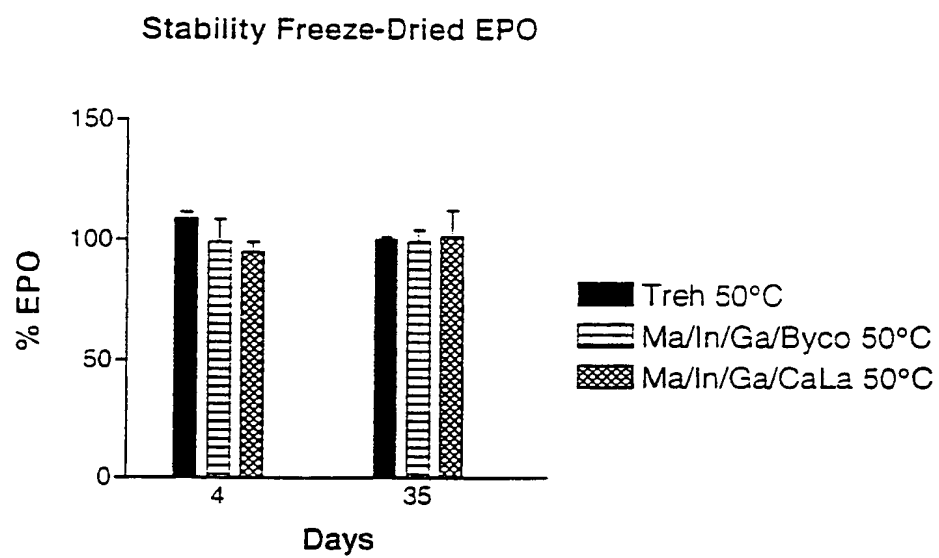
5/8

Figure 5



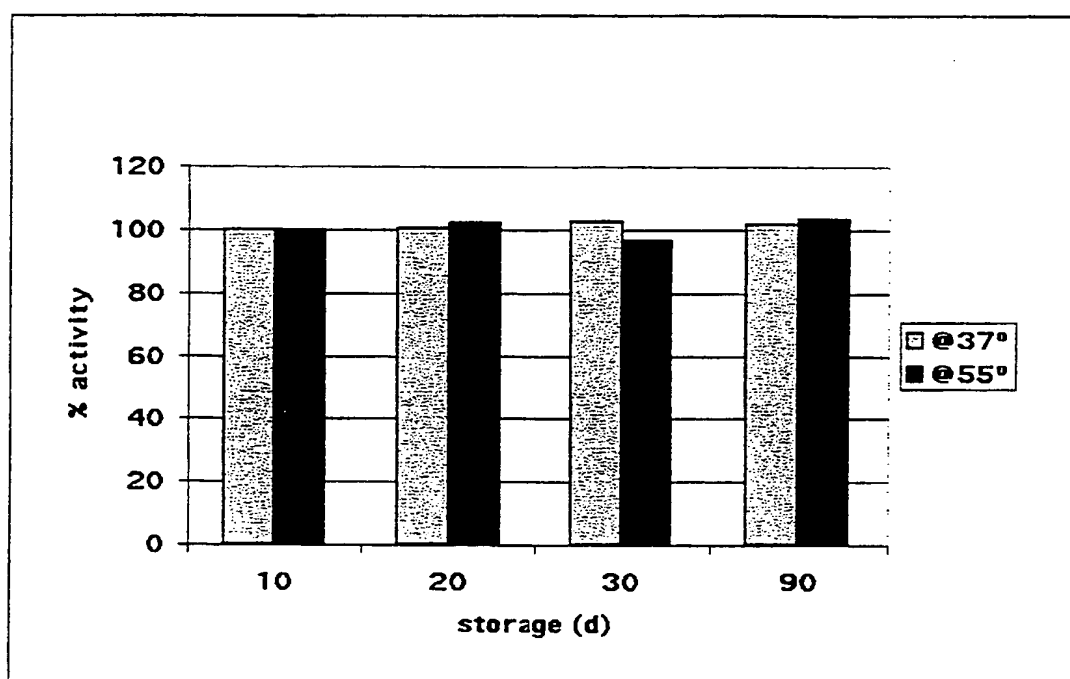
6/8

Figure 6



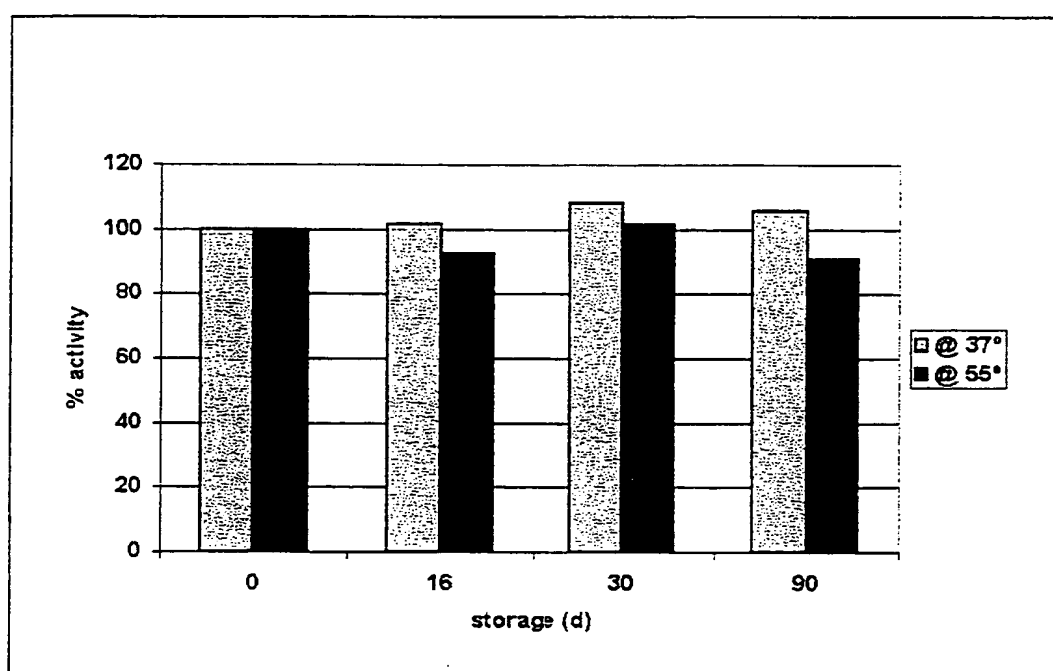
7/8

Figure 7



8/8

Figure 8



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/00820

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K47/26 A61K47/22 A23L1/275 A61K7/00 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 40077 A (GRIBBON ENDA MARTIN ;QUADRANT HOLDINGS CAMBRIDGE (GB); ROSER BRUCE) 19 December 1996 see abstract see page 5, line 9 - page 13, line 29 see examples 5,6 see claims 1-78 ---	1-11,13, 14
X	US 4 806 524 A (KAWAGUCHI TSUTOMU ET AL) 21 February 1989 see abstract see column 1, line 30 - line 40 see table see examples 1-11 --- -/--	1-11,14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 July 1999

Date of mailing of the international search report

06/08/1999

Name and mailing address of the ISA

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Authorized officer

Taylor, G.M.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 99/00820

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>EP 0 682 944 A (SANOFI SA) 22 November 1995 see abstract see page 3, line 31 - page 5, line 25 see examples 1-5 see claims 1-11 see figure 2</p> <p style="text-align: center;">---</p>	1-11, 14
X	<p>WO 96 05809 A (QUADRANT HOLDINGS CAMBRIDGE ;COLACO CAMILO (GB); ROSER BRUCE JOSEP) 29 February 1996 cited in the application see abstract see claims 1-13, 23-35</p> <p style="text-align: center;">---</p>	1-9, 13, 14
X	<p>US 5 621 094 A (ROSER BRUCE J ET AL) 15 April 1997 cited in the application see abstract see examples 1, 2 see claims 1-11</p> <p style="text-align: center;">---</p>	1-10
X	<p>US 5 589 167 A (CLELAND JEFFREY L ET AL) 31 December 1996 cited in the application see abstract see column 2, line 14 - line 39 see example II see claims 1-16</p> <p style="text-align: center;">---</p>	1-10
P, X	<p>WO 98 41188 A (SEN SHEVANTI DEVIKA ;EASTBRIDGE LIMITED (GB); ROSER BRUCE JOSEPH () 24 September 1998 see abstract see page 6, line 14 - page 7, line 20 see examples 1-3 see claims 1-23</p> <p style="text-align: center;">-----</p>	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/00820

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9640077	A	19-12-1996	AU 6009896 A CA 2223438 A CN 1193908 A CZ 9703912 A EP 0831790 A NO 975773 A PL 323902 A SK 167597 A	30-12-1996 19-12-1996 23-09-1998 13-05-1998 01-04-1998 03-02-1998 27-04-1998 07-10-1998
US 4806524	A	21-02-1989	JP 61097229 A CA 1258629 A EP 0178665 A	15-05-1986 22-08-1989 23-04-1986
EP 0682944	A	22-11-1995	FR 2719479 A AT 178484 T AU 694763 B AU 1777495 A CA 2148537 A CN 1116522 A CZ 9501081 A DE 69508837 D FI 952119 A HU 72325 A JP 8053361 A NO 951724 A NZ 272045 A PL 308416 A US 5763409 A ZA 9503596 A	10-11-1995 15-04-1999 30-07-1998 16-11-1995 05-11-1995 14-02-1996 14-02-1996 12-05-1999 05-11-1995 29-04-1996 27-02-1996 06-11-1995 27-02-1996 13-11-1995 09-06-1998 04-11-1996
WO 9605809	A	29-02-1996	AU 3349695 A CN 1160345 A EP 0804163 A JP 10505591 T	14-03-1996 24-09-1997 05-11-1997 02-06-1998
US 5621094	A	15-04-1997	AT 171209 T AU 7872591 A DE 69130223 D EP 0541556 A ES 2125237 T WO 9118091 A JP 5508315 T	15-10-1998 10-12-1991 22-10-1998 19-05-1993 01-03-1999 28-11-1991 25-11-1993
US 5589167	A	31-12-1996	US 5753219 A US 5804557 A AU 685784 B AU 6241294 A CA 2154164 A CN 1118143 A CZ 9502127 A EP 0686045 A JP 8507064 T NZ 262634 A WO 9419020 A ZA 9401239 A	19-05-1998 08-09-1998 29-01-1998 14-09-1994 01-09-1994 06-03-1996 14-02-1996 13-12-1995 30-07-1996 24-02-1997 01-09-1994 23-08-1995
WO 9841188	A	24-09-1998	AU 6510198 A	12-10-1998

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DAVIES, Jonathan Mark
REDDIE & GROSE
16, Theobalds Road
London WC1X 8PL
GRANDE BRETAGNE

File at Cambridge

JMD

RI

RECEIVED

9 JUN 2000

PCT
CAMBRIDGE

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing
(day/month/year)

06.06.2000

Applicant's or agent's file reference
39567 / JMD

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/00820

International filing date (day/month/year)
17/03/1999

Priority date (day/month/year)
18/03/1998

Applicant

RONAI, PETER et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

CAMBRIDGE

DUE DATE

18 / 9 / 00

INITIALS

MM

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Fax: +49 89 2399 - 4465

Authorized officer:

Spoke to MP + collected
ALL O.K.
Oberhauser, A

Tel. +49 89 2399-8139

INTERNATIONAL COOPERATION TREATY

CC BQ

RECEIVED

- 8 OCT 1999

PCT

CAMBRIDGE

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

File at Cambridge

DAVIES, Jonathan, Mark
Reddie & Grose
16 Theobalds Road
London WC1X 8PL
ROYAUME-UNI

JMD

RI

Date of mailing (day/month/year) 23 September 1999 (23.09.99)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 39567 / JMD	
International application No. PCT/GB99/00820	International filing date (day/month/year) 17 March 1999 (17.03.99)

1. The following indications appeared on record concerning:		
<input checked="" type="checkbox"/> the applicant	<input checked="" type="checkbox"/> the inventor	<input type="checkbox"/> the agent
<input type="checkbox"/> the common representative		
Name and Address CAMBRIDGE BIOSTABILITY LIMITED Sumper House 8 Station Road Histon Cambridge CB4 4LQ United Kingdom	State of Nationality GB	State of Residence GB
Telephone No.		
Facsimile No.		
Teleprinter No.		
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input checked="" type="checkbox"/> the person	<input type="checkbox"/> the name	<input type="checkbox"/> the address
<input type="checkbox"/> the nationality		
<input type="checkbox"/> the residence		
Name and Address RONAI, Peter 3621 Augusta National Drive S. Salem, OR 97302-9715 United States of America	State of Nationality US	State of Residence US
Telephone No.		
Facsimile No.		
Teleprinter No.		
3. Further observations, if necessary: the applicant in Box 1 has assigned all their rights to the application to the new applicant indicated in Box 2.		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input checked="" type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input type="checkbox"/> the elected Offices concerned	
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer F. Gateau
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

REC'D 09 JUN 2000

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 39567 / JMD	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB99/00820	International filing date (day/month/year) 17/03/1999	Priority date (day/month/year) 18/03/1998
International Patent Classification (IPC) or national classification and IPC A61K47/26		
Applicant RONAI, PETER et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 7 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 15/10/1999	Date of completion of this report 06.06.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Taylor, G.M. Telephone No. +49 89 2399 8406 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/00820

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-10 as originally filed

Claims, No.:

1-15 as originally filed

Drawings, sheets:

1/8-8/8 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 1.

because:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/00820

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 1 are so unclear that no meaningful opinion could be formed (*specify*):
- see separate sheet**
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	12,15
	No:	Claims	1-11,13,14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/00820

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Section I

1. The proposed amendments to the claims go beyond the content of the application as filed and therefore do not meet the requirements of Rule 70.2(c) PCT. Specifically, claims 1 and 7 have no basis or support in the application as filed and contain added subject-matter.

As a consequence of the above, this IPER is based upon the set of claims as originally filed.

Section III

2. The subject-matter of claim 1 is initially directed towards a method of drying, but attempts to characterise this in terms of a the method of preparation. In fact, the only feature of this claim which relates to drying is 'subjecting the composition to drying'.

Since the scope of the claimed subject-matter is only clear in a limited respect i.e. the production of the glasses of claims 7-15, the following Opinion relating to claims 1-6 and 10-15 has been restricted to a method of production of the glasses as defined in claims 7-15.

Section V

3. Claims 1-11, 13 and 14 do not meet the requirements of Art. 33(2) PCT because their subject-matter is not novel over the following documents:

**WO 96/40077 (D1); US-A-4 806 524 (D2); EP-A-0 682 944 (D3);
WO 96/05809 (D4); US-A-5 621 094 (D5); US-A-5 589 167 (D6).**

See the passages cited in the search report.

4. Claims 1-15 do not meet the requirements of Art. 33(3) PCT.
 - 4.1 The subject-matter of the claims cannot be seen as being inventive because, as is disclosed in, and indeed demonstrated by, the present application, the underlying technical problem is not solved over the whole scope of the claims.

It is disclosed on p.3, lines 25ff, that the teachings of **WO 96/05809 (D4)** are that mannitol has no stabilising effect. The description goes on to say that "certain monosaccharide sugar alcohols such as mannitol and inositol can be excellent stabilisers when correctly formulated".

As indicated in Item 1 (above), it is clear that the scope of the present claims overlaps with that of D3. Hence, if - as the Applicant states - correct formulation can render mannitol and inositol good stabilisers, then it is clear that essential features are missing from the claims (Rule 6.3(b)(i) PCT).

- 3.2 The fact that the technical problem is not solved over the whole scope of the claims is also demonstrated by Figures 4 and 6.

In Figure 4 it is clear that the best stability of freeze-dried alkaline phosphatase is obtained using trehalose alone (i.e. in the absence of the "glass-former") in comparison with the inclusion of a "glass-former", i.e. degraded gelatin (Byco C).

In Figure 6, there would appear to be no effect obtained by the addition of a "glass-former" (Byco C or calcium lactate).

It should also be noted that comparative tests which are intended to show an advantageous or unexpected effect must be made between the subject-matter of the application and the closest prior art. thus, since compositions containing trehalose alone are known from the prior art (e.g. D ...), the data in Figures 4 and 6 have been interpreted in this way.

- 3.3 Inventive step could be acknowledged for claims which are limited to subject-matter which has been shown to solve the underlying technical problem.
4. The subject-matter of claim 15 is also obvious because the optimisation of amounts is a matter of simple trial and error, as stated in the description on p.8, lines 23-28. Thus, in the absence of an unexpected or beneficial technical effect, these combinations cannot be seen as being inventive.

Section VI

5. The following document is cited under Rules 64.3 and 70.10 PCT:

WO 98/41188

Published on: 24.09.1998

Filed on: 18.03.1998

Priority: 18.03.1997

Section VIII

6. Claims 1, 7 and 10-15 do not meet the requirements of Art. 6 PCT.

- 6.1 In claims 1 and 7, the terms "glass-former" and "glass-formation-facilitator" are vague and unclear.

Furthermore, these claims define their subject-matter in terms of functional features/ a result to be achieved, rather than in terms of technical features, as required by Rule 6.3(a) PCT.

Thus, the following expressions/terms are considered unacceptable:

"a glass former or a glass-formation-facilitator" (functional feature: claims 1 and 7); and

"whereby the compound solidifies from solution as an amorphous class rather than by forming crystals" (result to be achieved: claim 1).

- 6.2 Claims 10-15 are unacceptable because the category of claim is unclear (PCT Guidelines C-III, 4.1).

- 6.3 Claim 15 is unacceptable because the use of trademarks (i.e. Byco C and Kollidon 30) should be avoided (PCT Guidelines C-III, 4.6a).



PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 39567 / JMD	FOR FURTHER ACTION		see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/GB 99/ 00820	International filing date (day/month/year) 17/03/1999	(Earliest) Priority Date (day/month/year) 18/03/1998	
Applicant CAMBRIDGE BIOSTABILITY LIMITED et al.			

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

AMORPHOUS GLASSES FOR STABILISING SENSITIVE PRODUCTS

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

Box III TEXT OF THE ABSTRACT (Continuation of Item 5 of the first sheet)

Disclosed is a method of drying, without damage, a compound which is subject to deactivation on drying, or a mixture of such compounds, comprising subjecting an aqueous system containing the compound or mixture to drying in the presence of one or more monosaccharide sugar alcohols and at least one additive which is a glass-former or a glass-formation-facilitator, whereby the compound solidifies from solution as an amorphous glass rather than by forming crystals.

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VAT No. GB 243 9087 49

URGENT

FACSIMILE TRANSMISSION

Date : 27 March 2000

To : EPO MUNICH

For The Attention Of : IPEA

Your Ref : PCT/GB99/00820 : Ronald, Peter et al.;

Our Ref : 39567/PCT/JMD

Number of pages : 1 plus this cover sheet

(X) Please advise immediately if any pages are not received

() Please acknowledge receipt of this transmission by return

Further Information :

URGENT

I refer to the Response filed on 17th March to the Written Opinion of 20th December 1999.

A typo has been noted in page 2 para 2; "polysaccharide" should read "monosaccharide" as shown in the attached replacement page. Please ensure that this correction is taken into account by the Examiner.

Davies, J M , Authorised Representative.

JD Davies

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If you are not the intended recipient, please do not pass it on
to anyone else, and let us know by return.

European Patent Office,
D-80298 Munich,
GERMANY.
Attn: International Preliminary Examining Authority

17 March 2000
Our Ref: JMD/JS/39567

Dear Sirs,

International Patent Application No. PCT/GB99/00820
Ronai, Peter *et al*

I am filing herewith a response to the Written Opinion dated 20th December 1999.

Firstly, I am filing herewith an amended set of claims. In particular, claim 1 has been amended to define a method for producing a dried product (for example the dried product of claim 7 *et seq*) and this method claim clearly indicates the various method steps including a step of drying and a step of solidifying, with the result that a dried product is produced in the form of an amorphous sugar glass which contains the other ingredients of the composition.

Secondly, the claimed invention no longer covers a method in which freeze drying is used. This distinguishes it from most of the cited prior art. Furthermore, the dried product of the method does not contain a crystalline phase as well as the glass phase.

As pointed out in the applicant's comments (attached hereto) the invention requires the use of at least three components in the aqueous system from which the dried product is produced: namely a monosaccharide sugar alcohol which does not normally form a glass on drying from solution, an active ingredient compound, and an additive which causes the sugar to form an amorphous glass rather than a crystal structure.

The advantage of the invention is, at least in part, the ability to use monosaccharide sugar alcohols (such as mannitol) as stabilisers, which sugar alcohols are widely accepted for industrial and pharmaceutical uses in contrast to the use of polysaccharide sugars (such as trehalose). Thus the invention allows the use of hitherto known but unused sugar alcohols to provide novel stabilised products. In the applicant's own description it is stated that certain monosaccharide sugar alcohols can be excellent stabilisers when correctly formulated. In its broadest form the invention resides in the teaching that a normally non-glass-forming sugar can be turned into a glass-forming sugar by the addition of a glass-former or a glass-formation-facilitator which changes the behaviour of the sugar and allows it to be used as a stabiliser. Not only is such an advantageous use not disclosed in the prior art, but the prior art explicitly teaches away from it.

The applicant has shown that a number of different compounds can be used as the glass-formation-facilitating additive. Those of ordinary skill in the art, taking the teaching of the present description, would be able to optimise the relative proportions of additives in order to ensure that the monosaccharide sugar alcohol or alcohols do not crystallise but form an amorphous glass.

The technical problem can be seen to be the provision of alternatives to stabilisation by polysaccharides such as trehalose. However, a direct comparison between the stabilisation achieved with trehalose and the stabilisation achieved according to the invention should not be seen as casting doubt on the validity and utility of the invention as defined. It is important to note that without the benefit of the present invention ~~polysaccharide~~ ^{monosaccharide} sugar alcohols such as mannitol would be expected (as taught by the prior art) to give no stabilisation whatsoever and consequently the ability to use mannitol and the like for stabilisation is in itself an immense technical contribution to the art. The ability to use sugars such as mannitol in formulations of the same general type as those using trehalose (whether the results are actually comparable or not) is a significant surprise to those of skill in this art who have based their previous expectations on the published documents cited by the Examiner. X

The applicant has added new claims (fully supported by the description) which are directed specifically to the currently preferred embodiments which have mannitol together with a glass-forming-facilitator chosen from either borate ions or calcium lactate.

A further advantage of the present invention is that a number of different monosaccharide sugar alcohols can be combined in order to raise the overall sugar concentration in the aqueous solution, and these monosaccharide sugar alcohols can be caused to form a glass by the addition either of a relatively small but effective amount of a glass-formation-facilitator or by the addition of a number of glass-formation-facilitating additives each of which being in an amount less than the "threshold amount" needed to cause glass formation individually. This gives significant flexibility in the way that products of the invention can be formulated.

Previous claim 15 (now claim 18) does, indeed, show a number of "optimised" formulations. The Examiner is urged to realise that without the benefit of the teaching of the present application those of skill in the art would not have expected such formulations to produce useful stabilising amorphous sugar glasses. On the contrary, the teaching of the prior art would have led those of skill in the art to have expected to have formed non-stabilised crystalline products from the compositions of the claim. This clearly demonstrates the unexpected and beneficial technical effect of the formulations disclosed in the present invention.

With regard to the prior application WO 98/41188, it is noted that this merely mentions in passing that mannitol might be used for stabilisation of a biomolecular product but does not explicitly describe the method or the dried product formulation according to the present invention. The current claims of the present invention are entitled to the priority dates of 18th March 1998 and 23rd September 1998 (i.e. prior to the publication date of 24th September 1998 of WO 98/41188) and since the claims of the present application are novel both in terms of the method steps and the formulation of the dried product it is submitted that the prior application is not relevant.

As will be deduced from the comments and arguments above, it is believed that the terms "glass-former" and "glass-formation-facilitator" are neither vague nor unclear in the context of the invention which relates to a means for causing a monosaccharide sugar alcohol to form a glass under conditions in which it would not normally form a glass (i.e. under conditions in which it would normally crystallise).

The Examiner's comment under section 6.2 with regard to claims 10 to 15 is noted: however the applicant has chosen to retain the form or wording "method or product" in order to keep the claims concise and to avoid simply repeating all of the claims as separate method claims and product claims.

With regard to the comments of the Examiner under section 6.3, the descriptions which have been objected to have been retained since these are well understood ways of describing particular components and any necessary acknowledgment of trade marks can be dealt with in the national/regional phases (for those territories in which the trade marks are registered).

It is believed that these comments and amendments should allow the Examiner to issue a positive International Preliminary Examination Report.

Yours sincerely,

Dr. J.M. Davies

ANNEX

GENERAL COMMENTS

The majority of the documents cited by the Examiner cover the use of glass forming substances to stabilise, and use substances which naturally form glasses on drying. They are rather difficult to crystallise. It is their glass forming ability which caused them to be selected as potential stabilisers in the first place. The glass formers they use are single substances which automatically form glasses on drying. This is the case with the Quadrant patents. A general description of the type of stabilising polyols which was thought to work is given in Quadrant's US patent 5,621,094 (D5) column 1, lines 49-57 and in tables 1a and 1b which also specify that the monosaccharide alcohols do not work. Our patent, in sharp contrast, uses sugars which to not normally form glasses and have been discounted in the past as being of any use as stabilisers; the monosaccharide sugar alcohols. The patentable discovery is that these substances are excellent stabilisers only when formulated in certain complex mixtures (not as a single substance). The unifying theme about these other components in the mixtures is that they are glass-forming-facilitators which inhibit the crystallisation of the monosaccharide sugar alcohols and force them to dry as glasses against their natural tendency to crystallise.

These glass facilitating substances can differ widely one from another. As an example, proteins such as gelatin and albumin work but only in concentrations similar to that of the mannitol. The same is true of other glass forming sugars such as trehalose or palatinit. These substances do not react chemically with the mannitol, but presumably promote glass formation by physico-chemical processes, possibly by increasing the viscosity to the point at which crystallisation could begin. Borates, on the other hand are effective in amounts less than one tenth of the amount of mannitol and react chemically with the mannitol to give stoichiometric amounts of mannitoborate. There is also a hydrogen bonded network created, by interaction of oxygen atoms in the borate ion with hydrogen atoms in the sugar, which dramatically inhibits crystallisation. Why this is so is unknown but there is a significant increase in the viscosity of the mixture, which could play a part.

It is also important that Mannitol is widely used as an excipient even though it crystallises in most formulations. This is mainly because it is an excellent bulking agent and the crystals aid in the formation of a good stable plug in freeze dried preparations. The crystalline mannitol may also facilitate the loss of water from the frozen plug by increasing its porosity. In this sense it helps to "stabilise" the formulation but this stabilising effect is minor and only of value with essentially stable drugs. Such use of crystalline mannitol should not be confused with the invention which can provide an amorphous mannitol glass to stabilise an "unstable" drug.

Several patents quoted against us are on freeze-drying using mannitol eg D2 (US 4,806,524 CHUGAI), D3 (EP 0682944 US5,763,409 SANOFI), EP 0438747 SHIONOGI, and D6 US5,589,167 GENENTECH).

Also the data we show in Figs 4 and 6 shows better preservation with trehalose than with the mannitol glasses in the freeze dried preparations.

Also freeze drying with trehalose is in the public domain. For these three reasons we are amending our application to exclude freeze drying. The drying methods according to the invention still cover spray, vacuum and air drying at temperatures above freezing.

SPECIFIC CITATIONS

D1 (WO96/40077) "Methods for stably incorporating substances within dry Foamed Glass matrices and compositions obtained thereby"

This patent claims the use of "Stabilising Polyols" and lists the preferred ones in claim 6. Mannitol and the other monosaccharide alcohols used in our patent are not listed because they had previously been shown, in the other patents discussed in our patent and in the references quoted on page 2, lines 20-24, to be non-stabilising. We also differentiate our patent on the basis that the sugar alcohols used in our invention do not normally form glasses and hence they would not be expected to be useful in the formation of foamed glass matrices as taught by D1.

D2 (US4,806,524) "Stable erythropoietin preparations and process for formulating the same" (Chugai)

This patent refers only to freeze-drying. Also it tests stability only at 37°C for one month; a very mild stress which defines stabilisation as a minor protection against environmental stress. Our product can be 100% stable at 50°C, at which temperature even the trehalose-stabilised product had lost 80-90% of its activity (figs. 3 and 5).

D3 (EP-A-0 682 944) "Stable freeze-dried formulations comprising a protein assay kit" (Sanofi)

Again refers only to freeze-drying. This one also defines stability at very feeble stress viz. 35°C for one month. However they do show that the mannitol is made into a glass by excess alanine up to a mannitol/alanine ratio of 1/1 and that it is the mannitol glass that is stabilising. We exclude freeze-drying. Also the alanine itself crystallises, which we exclude. The prior art explicitly states that the "amorphous phase predominantly consists of mannitol and protein" and the "crystalline phase predominantly consists of alanine" (D3: page 3, lines 36-42).

D4 (WO 96/05809) "Stabilisation of biological macromolecular substances and other organic compounds" (Quadrant)

This patent actually specifically excludes mannitol and other monosaccharide alcohols eg in Table 1a and on page 7 line 26 to page 8 line 6:-

"Thus the monosaccharide sugar alcohols galactitol, mannitol and erythritol are not satisfactory protective agents. This is not due to the fact that a ring structure is required for stabilising activity. Sorbitol (glucitol) a straight chain non-reducing monosaccharide alcohol does have some limited activity as a stabilising agent while myo-inositol a non-reducing 6 carbon ring compound is without stabilising activity."

This is all dealt with on page 3 of our application.

D5 (US 5,621,094) "Method of preserving agarose gel structure during dehydration by adding a non-reducing glycoside of a straight chain sugar alcohol" (Quadrant)

This patent actually specifically excludes mannitol and other monosaccharide alcohols eg in Table 1a and on column 1 line 53 and in particular column 3 lines 25 - 33:-

"Absence of a reducing group is not sufficient to guarantee stabilizing ability. Thus the monosaccharide sugar alcohols galactitol, mannitol and erythritol are not satisfactory protective agents. This is not due to the fact that a ring structure is required for stabilising activity. Sorbitol (glucitol) a straight chain non-reducing monosaccharide alcohol does have some limited activity as a stabilising agent while myo-inositol a non-reducing 6 carbon ring compound is without stabilising activity."

This is all dealt with on page 3 of our application.

**D6 (US 5,589,167) "Excipient stabilization of polypeptides treated with organic solvents"
(Genentech)**

We specifically address this patent in detail in our patent application on page 5. Again the Cleland patent specifies freeze-drying. However the points made in our original application can differentiate it from ours. Namely, that the Cleland patent demands that large amounts of the active proteins and small amounts of mannitol or trehalose are used so that the active prevents crystallisation of the mannitol by acting as a glass former itself. Our patent forms a glass of the mannitol. This enables products to be made that contain only small amounts ie single doses of very potent actives.